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(71) Applicant *(for all designated States except US)*: EUGENE SCIENCE INC. [KR/KR]; 6th Floor, Namgang Building, 162-4, Dongkyo-dong, Mapu-gu, Seoul 121-200 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NOH, Seung-Kwon [KR/KR]; C-211 Jingu Apt., Yoido-dong, Youngdungpo-gu, Seoul 150-010 (KR). CHUNG, Dae-Won [KR/KR]; 102-103 Dusandong Apt., 1194-1, Kwonsun-dong, Kwonsun-gu, Suwon, Kyungy 441-390 (KR).

(74) Agent: CHOI, Hong-Soon; Hosan Plaza, Suite 305, 37 Samsung-dong, Kangnam-gu, Seoul 135-090 (KR).

(54) Title: PROCESS FOR REDUCING THE CONTENT OF CHOLESTEROL IN DAIRY PRODUCTS

(57) Abstract

A process for removing cholesterol contained in an emulsion of animal origin having fat globules includes the step of reducing the size of the fat globules of the emulsion to a predetermined size. The process also includes the step of contacting the emulsion with a predetermined amount of cyclodextrin such that the cyclodextrin forms insoluble inclusion complexes with the cholesterol and the step of separating substantially all of the complexes from the emulsion. The predetermined size of the fat globules is within a range sufficient to cause the removal of substantially all of the cholesterol from the emulsion in a single performance of the contacting step and the separating step.

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PROCESS FOR REDUCING THE CONTENT OF CHOLESTEROL IN DAIRY PRODUCTS

Technical Field of the Invention

The present invention relates to a process for reducing the content of cholesterol in food products and, more particularly, to a process for reducing the content of cholesterol in dairy products, such as milk.

Background Art

Because of health risks associated with the consumption of food products having a high cholesterol content, various processes have been developed, in the past, for reducing the cholesterol content in food products. Some of these processes utilize cyclodextrin (see, for instance, U.S. Patent Nos. 4,880,573; 5,063,007; 5,223,295; 5,264,226; 5,264,241; 5,292,546; 5,232,725; 5,342,633; 5,484,624; and 5,498,437). More particularly, cyclodextrin is believed to have a selective affinity to cholesterol present, for instance, in fat globules of milk. Accordingly, when added to milk, the cyclodextrin combines with cholesterol and forms insoluble cholesterol-cyclodextrin inclusion complexes. The cholesterol-cyclodextrin inclusion complexes are then removed from the milk by using a conventional separation method (e.g., filtration and centrifugation) to yield milk with a reduced cholesterol content.

It is believed that the foregoing conventional processes using cyclodextrin are poor, inadequate and/or inefficient for use in producing milk having an extremely low cholesterol content. For instance, in accordance with the process disclosed in U.S. Patent No. 5,264,226, the content of cholesterol in the cream of a

milk can be reduced by about 52% by treating the milk with β-cyclodextrin (see Example 1 of the '226 Patent). In order to reduce the cholesterol content further (i.e., to obtain a greater than 90% overall reduction), the milk needs to be re-processed. That is, the milk needs to be re-treated with another application or treatment of cyclodextrin, thereby rendering the process inefficient. In addition, it has been observed that when milk is repeatedly treated with cyclodextrin, its taste and nutritive values are significantly compromised.

Disclosure of the Invention

The present invention overcomes the disadvantages and shortcomings of the prior art discussed above by providing a new and improved process for removing cholesterol contained in an emulsion of animal origin having fat globules. More particularly, the process includes the step of reducing the size of substantially all of the fat globules to a predetermined size. The process also includes the steps of contacting the emulsion with a predetermined amount of cyclodextrin such that the cyclodextrin forms insoluble inclusion complexes with the cholesterol, and separating substantially all of the complexes from the emulsion. The predetermined size is within a range sufficient to cause the removal of substantially all of the cholesterol from the emulsion in a single performance of the contacting step and the separating step.

In accordance with one feature of the present invention, the process includes the step of determining whether substantially all of the fat globules have the predetermined size. If the size of substantially all of the fat globules is not

determined to be within the range of the predetermined size, the reducing step is re-performed or repeated so as to reduce the size of fat globules to the predetermined size.

In accordance with another feature of the present invention, the process includes the step of selecting, as a starting material, an emulsion of animal origin having fat globules, substantially all of which have the predetermined size. In such circumstances, the reducing step may be omitted.

Best Mode for Carrying Out the Invention

Although the present invention can be used in conjunction with any type of oil-in-water emulsion of animal origin, it is particularly suitable for use in connection with milk. Accordingly, the present invention will be described hereinafter particularly in connection with milk. It should be understood, however, that the following description is only meant to be illustrative of the present invention and is not meant to limit the scope of the present invention, which has applicability to other types of oil-in-water emulsions.

The present invention involves a cholesterol reducing process for reducing the content of cholesterol in various dairy products (e.g., milk, yogurt and cream). The cholesterol reducing process includes a pre-treatment or preparation step of pre-treating starting milk (i.e., milk used as a starting material) and a removing step of removing cholesterol from the pre-treated milk (i.e., the milk resulting from the pre-treatment step) with the use of β-cyclodextrin. More particularly, during the pre-treatment step, the starting milk is processed so that fat

globules contained therein are reduced (i.e., broken up) to a small, relatively uniform size. That is, substantially all of the fat globules are reduced to a predetermined size of preferably no greater than about 3 μm, more preferably from about 0.1 μm to about 3 μm and most preferably from about 0.1 μm to about 2 μm and/or to a predetermined mean size of preferably no greater than about 0.99 μm, more preferably from about 0.76 μm to about 0.99 μm and most preferably about 0.76 μm.

Applicant has, quite surprisingly and unexpectedly, demonstrated that when the fat globules of the starting milk are reduced to the foregoing predetermined size and/or predetermined mean size, substantially all (e.g., about 98%) of the cholesterol contained in the starting milk can be removed in a single operation or cycle of the cholesterol removing process of the present invention. According to the present invention, the fat globules of the starting milk can be reduced to sizes much smaller than the predetermined size and/or the predetermined mean size mentioned above (e.g., less than 0.1 μm). However, because high energy is required in producing smaller sized fat globules and because such fat globules are not believed to provide substantially improved results, it would not be desirable to pre-treat the starting milk such that the fat globules are reduced to a size much less than the predetermined size and/or the predetermined mean size.

While any conventional processes can be used for reducing the fat globules to the predetermined size and/or the predetermined mean size described above, those used in the milk processing field to homogenize raw milk with the use of pressure are preferred. Other conventional methods suitable for use in connection with the pre-treatment step include sonification.

In the milk processing field, homogenization processes are typically performed using a wide range of pressures, resulting in milk having widely varying fat globule sizes. In performing the pre-treatment step of the present invention using a conventional homogenization process, applicant has demonstrated that the fat globules of the starting milk are reduced to the predetermined size and/or the predetermined mean size by using a pressure of preferably not less than about 100kg/cm^2, more preferably from about 100 kg/cm^2 to about 200 kg/cm^2 and most preferably about 200 kg/cm^2. Accordingly, the starting milk is preferably heated to about 40°C and is pressurized to the foregoing pressure. The pressurized milk is then sprayed to break up the fat globules into small fat globules having a size substantially equal to the predetermined size and/or the predetermined mean size.

The starting milk can be any type of milk. For instance, the milk can be raw, homogenized, unhomogenized, heat-treated (e.g., pasteurized or sterilized) or enriched in fat. Further, the starting milk can have any fat globule sizes. Other types of oil-in-water emulsions of dairy or animal origin, such as cream, can also be used as a starting material.

After the pre-treatment step, the size of the fat globules of the pre-treated milk can be measured or calculated in order to ensure that it is within the range of the predetermined size and/or the predetermined mean size mentioned above. In performing this measuring step, any conventional methods for measuring or calculating the size of microscopic particles can be used. For instance, the size of the fat globules can be measured and/or calculated by using a conventional optical microscope. If an optical microscope is used, a sample of the pre-treated milk

is placed on the optical microscope, and the optical microscope magnification is adjusted to a desired setting. A fat globule image is then taken by using a "CCD" camera. Next, the image taken by the camera is processed by an image analyzer to filter out images of particles other than fat globules. Thereafter, the size of the fat globules in the processed image is calculated in accordance with the magnification setting of the optical microscope in a conventional manner. If the size of the fat globules is determined to be within the range of the predetermined size and/or the predetermined mean size, the pre-treated milk is ready for the removing step (i.e. the removing step is performed with the pre-treated milk). If the size of the fat globules is not within the range of the predetermined size and/or the predetermined mean size, the milk is re-processed (i.e., the pre-treatment step is repeated), preferably using a pressure higher than the one used in the previous pre-treatment step.

While the measuring step is preferably carried out after the pre-treatment step, it can be performed prior to same in order to determine whether the pre-treatment step is necessary. More particularly, if the fat globule size of the starting milk is determined to be within the range of the predetermined size and/or the predetermined mean size, the performance of the pre-treatment step is unnecessary. In such circumstances, the removing step is performed immediately after the measuring step without performing the pre-treatment step (i.e., the pre-treatment step is skipped). The measuring step can also be performed both prior to and after the pre-treatment step to ensure that the fat globules are properly reduced to the predetermined size and/or the predetermined mean size.

After determining that the size of the fat globules of the pre-treated milk is within the range of the predetermined size and/or the predetermined mean size, the removing step is performed. The removing step of the present invention can be carried out by using any conventional processes, such as the one disclosed in U.S. Patent No. 5,264,226, the specification of which is incorporated herein by reference. For instance, β-cyclodextrin is added to the pre-treated milk. Alternatively, other types of cyclodextrin (e.g., α-cyclodextrin) can be used. In addition, while any form of β-cyclodextrin can be used, β-cyclodextrin in powder form is preferred. The concentration of β-cyclodextrin is preferably between 0.5 and 5 (w/v)% and more preferably about 1 (w/v)%.

After adding β-cyclodextrin to the pre-treated milk, the milk-cyclodextrin mixture is rigorously mixed (i.e., the pre-treated milk is contacted by β-cyclodextrin) so as to facilitate the formation of insoluble cholesterol-cyclodextrin inclusion complexes. More particularly, the milk-cyclodextrin mixture is mixed preferably at about 0 °C to 20°C for about 5 to 30 minutes by using a stirrer rotating at about 50 to 500 r.p.m. The cholesterol-cyclodextrin inclusion complexes are then removed from the milk by centrifuging the mixture using a centrifuge rotating preferably at about 2,000 to 6,000 r.p.m. for about 1 to 5 minutes. The centrifugation temperature of the milk is preferably from 4°C to 25°C. Alternatively, other suitable separation methods, such as decantation and filtration, can be used to remove the cholesterol-cyclodextrin inclusion complexes from the milk.

It should be appreciated that the resultant milk (i.e., the milk resulting from the cholesterol reducing process of the present invention) is substantially free

of cholesterol. More particularly, as will be discussed further hereinafter, the process of the present invention removes at least about 98% of the cholesterol contained in the starting milk. That is, the process of the present invention yields a cholesterol reduction ratio (i.e., a ratio between the amount of cholesterol removed from the starting milk and the original amount of cholesterol present in the starting milk) of about 98% or more.

Because the present invention yields a cholesterol reduction ratio of at least about 98% in a single performance of the removing step (i.e., by a single application of cyclodextrin to the starting milk), it is not necessary to re-process the resultant milk with another application or treatment of β-cyclodextrin in order to remove additional cholesterol therefrom, thereby rendering the process of the present invention efficient and effective. In addition, because the resultant milk is not subject to another β-cyclodextrin treatment, the original taste and nutritive values of the starting milk are substantially preserved in the resultant milk. More particularly, the taste and nutritive values associated with milk are believed to be correlated to the concentrations of milk fat and milk protein present therein. It has been observed that the concentrations of milk fat and milk protein in the resultant milk according to the present invention is comparable to those of non-processed milk (i.e., milk without a cyclodextrin treatment). In contrast, as noted above, the prior art processes require repeated cyclodextrin treatments to achieve a relatively high overall reduction ratio. Because repeated cyclodextrin treatments are believed to have a significantly detrimental impact on milk fat and milk protein concentrations, the prior art processes are poor in preserving the original taste and nutritive values of milk.

The foregoing results of the present invention have been, quite surprisingly and unexpectedly, demonstrated only after conducting a whole set of tests and studies. More particularly, in performing initial tests and studies, applicant mixed β-cyclodextrin to homogenized milk and unhomogenized milk without specifically performing the pre-treatment step of the present invention. It was surprisingly and unexpectedly observed that the homogenized milk used as a starting material during these initial tests and studies yielded an extremely low cholesterol content, while the unhomogenized milk yielded milk having a relatively high cholesterol content. To facilitate consideration and discussion, the results of these initial tests and studies are provided in Examples 1-6 hereinafter. Upon further tests and studies, applicant has, more surprisingly and unexpectedly, demonstrated that when the fat globules of the milk used as a starting material for the removal step of the present invention have a size equal to the predetermined size and/or the predetermined mean size, the content of cholesterol can be reduced by at least about 98%.

In accordance with the present invention, the cholesterol reducing process can have many variations and modifications. For instance, cyclodextrin can be added to the milk prior to the pre-treatment step. More particularly, cyclodextrin can be added directly to the starting milk (i.e., the milk used as a starting material of the cholesterol reducing process of the present invention) prior to the performance of the pre-treatment step. The milk-cyclodextrin mixture can then be processed under a conventional homogenization process as described above to reduce the size of the fat globules to the predetermined size and/or the predetermined mean size.

The pre-treatment step of the present invention may be omitted by selecting milk having fat globules which have a size within the range of the predetermined size and/or the predetermined mean size. More particularly, if such milk is selected as a starting material for the cholesterol removing process of the present invention, it may not be necessary to specifically perform the pre-treatment step.

It should also be noted that the cholesterol reducing process of the present invention can be performed in a single, continuous operation. In such circumstances, the cholesterol reducing process of the present invention can be incorporated into conventional milk-processing methods, dairy product manufacturing processes and the like. Accordingly, the resultant milk of the present invention can be further processed in a conventional manner to yield various dairy products having an extremely low cholesterol content. For instance, the resultant milk can be further processed so as to be separated into cream and skim milk, thereby resulting in cream and skim milk having a low cholesterol content.

The following examples further illustrate the present invention. In this regard, it should be noted that these examples are not meant to limit the scope of the present invention. It is also noted that in the following examples, the original amount of cholesterol contained in the starting milk is 12.6 mg per 100 g of milk.

COMPARATIVE EXAMPLE 1

To 100 liters of unhomogenized milk, 1(w/v)% of β-cyclodextrin in powder form is added. The milk-cyclodextrin mixture is then mixed at 500 r.p.m. at

the temperature listed in Table 1 (see below) for the time period indicated therein so as to facilitate the formation of insoluble cholesterol-cyclodextrin inclusion complexes. The milk-cyclodextrin mixture is then centrifuged at 4°C for 10 minutes at 3,000 r.p.m. to separate the cholesterol-cyclodextrin inclusion complexes from the milk. As a result, three layers (i.e. a cream portion, a skim milk portion and a cholesterol-cyclodextrin inclusion complex portion) are formed. After discarding the cholesterol-cyclodextrin inclusion complex portion, the skim milk portion and the cream portion are re-mixed (e.g., homogenized). The resultant milk is then checked for its cholesterol content via the Carr Drekter method using Libermann Buchard reaction. The cholesterol content is listed in Table 1 along with its corresponding reduction ratio.

TABLE 1

Mixing Temp. (°C)	Mixing Time (Min)							
	5		10		15		30	
	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)
4	10.38	17.6	9.64	23.5	9.26	26.5	9.17	27.2
7	10.18	19.2	9.19	27.1	9.19	27.1	9.26	26.7
20	10.66	15.4	10.33	18.0	10.36	17.8	10.18	19.2
40	11.57	8.2	10.99	12.7	10.94	13.2	11.03	12.5

As indicated in Table 1, when unhomogenized milk is used as a starting material for the removing step without performing the pre-treatment step of the present invention, poor results are obtained. That is, the reduction ratio obtained with respect to the unhomogenized milk is no greater than 28%.

EXAMPLE 2

To 500 liters of homogenized milk, powdered β-cyclodextrin in the amount listed in Table 2 (see below) is added. The milk-cyclodextrin mixture is then mixed at 300 r.p.m. for 10 minutes at the temperature listed in Table 2 so as to facilitate the formation of insoluble cholesterol-cyclodextrin inclusion complexes. Next, the milk-cyclodextrin mixture is centrifuged at 2,000 r.p.m. for 5 minutes at 4°C to separate the cholesterol-cyclodextrin inclusion complexes from the milk. After discarding the cholesterol-cyclodextrin inclusion complexes and mixing (e.g., homogenizing) the cream portion with the skim milk portion, the resultant milk is checked for its cholesterol content using the method mentioned in Example 1. The cholesterol content is listed in Table 2 along with its corresponding cholesterol reduction ratio.

TABLE 2

Mixing Temp. (°C)	0.5 (w/v)% β-cyclodextrin		1 (w/v)% β-cyclodextrin		2 (w/v)% β-cyclodextrin	
	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)
4	2.12	83.2	0.92	92.7	1.27	89.9
7	1.75	86.1	0.86	93.2	1.26	90.0
20	1.34	89.4	1.16	90.8	1.74	86.2

As indicated in Table 2, the homogenized milk used in Example 2 as a starting material yields surprising and unexpected results compared to the results obtained in connection with the unhomogenized milk of Example 1. That is, the reduction ratio associated with the homogenized milk is much greater than the reduction ratio associated with the unhomogenized milk of Example 1. It can also

be observed in Table 2 that when 1(w/v)% of β-cyclodextrin is added to the homogenized milk, the mixing temperature does not have a significant impact on the reduction ratio.

EXAMPLE 3

To 100 liters of homogenized milk, powdered β-cyclodextrin in the amount listed in Table 3 (see below) is added. The milk-cyclodextrin mixture is then mixed at 500 r.p.m. for 10 minutes at 4°C. Next, the milk-cyclodextrin mixture is centrifuged at 6,000 r.p.m. for 1 minute at the temperature listed in Table 3. After discarding the cholesterol-cyclodextrin inclusion complexes and mixing the cream portion with the skim milk portion, the resultant milk is checked for its cholesterol content using the method mentioned in Example 1. The cholesterol content is listed in Table 3 along with its corresponding cholesterol reduction ratio.

TABLE 3

Centrifugation Temp. (°C)	0.5 (w/v)% β-cyclodextrin		1 (w/v)% β-cyclodextrin		2 (w/v)% β-cyclodextrin	
	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)
4	2.12	83.2	0.92	92.7	1.27	89.9
7	1.75	86.1	0.86	93.2	1.26	90.0
20	1.34	89.4	1.16	90.8	1.74	86.2
25	0.97	92.3	0.79	93.7	0.54	95.7

As indicated in Table 3, substantially constant results are obtained when the centrifugation temperature is varied between 4°C and 25°C. As a result, in the range from 4°C to 25°C, the centrifugation temperature does not have a significant impact on the reduction ratio.

EXAMPLE 4

To 100 liters of homogenized milk, powdered β-cyclodextrin in the amount listed in Table 4 (see below) is added. The milk-cyclodextrin mixture is then mixed at 500 r.p.m. for 10 minutes at 4°C. Next, the milk-cyclodextrin mixture is centrifuged at 6,000 r.p.m. for the time period listed in Table 4 at 4°C. After discarding the cholesterol-cyclodextrin inclusion complexes and mixing the cream portion with the skim milk portion, the resultant milk is checked for its cholesterol content using the method mentioned in Example 1. The cholesterol content is listed in Table 4 along with its corresponding cholesterol reduction ratio.

TABLE 4

Centrifugation Time (Min)	0.5 (w/v)% β-cyclodextrin		1 (w/v)% β-cyclodextrin		2 (w/v)% β-cyclodextrin	
	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)
1	0.67	94.7	1.27	89.9	0.19	98.5
2	0.08	99.4	0.05	99.6	0.04	99.7
5	0.16	98.7	0	100.0	0	100.0

As indicated in Table 4, a centrifugation time of 2 to 5 minutes is preferred. That is, when the milk-cyclodextrin mixture is centrifuged for 2 to 5 minutes, improved results are obtained.

EXAMPLE 5

To 100 liters of homogenized milk, powdered β-cyclodextrin in the amount listed in Table 5 (see below) is added. The milk-cyclodextrin mixture is then mixed at 500 r.p.m. for 10 minutes at 4°C. Next, the milk-cyclodextrin mixture is

centrifuged for 1 minute at the r.p.m. speed listed in Table 5. After discarding the cholesterol-cyclodextrin inclusion complexes and mixing the cream portion with the skim milk portion, the resultant milk is checked for its cholesterol content using the method mentioned in Example 1. The cholesterol content is listed in Table 5 along with its corresponding cholesterol reduction ratio.

TABLE 5

Speed of Centrifugation (r.p.m.)	0.5 (w/v)% β-cyclodextrin		1 (w/v)% β-cyclodextrin	
	Final Content (mg/100g)	Reduction Ratio (%)	Final Content (mg/100g)	Reduction Ratio (%)
2,000	2.75	78.2	1.36	89.2
4,000	2.36	81.3	1.6	87.3
6,000	2.2	82.5	1.41	88.8

As indicated in Table 5, in the range of 2,000 to 6,000 r.p.m., the centrifugation speed does not have a significant impact on the cholesterol reduction ratio.

EXAMPLE 6

To 100 liters of homogenized milk, powdered β-cyclodextrin in the amount listed in Table 6 (see below) is added. The milk-cyclodextrin mixture is then mixed at 500 r.p.m. for 10 minutes at 4°C. Next, the milk-cyclodextrin mixture is centrifuged at the centrifugation speed listed in Table 6 for 5 minutes at 4°C. After discarding the cholesterol-cyclodextrin inclusion complexes and mixing the cream portion with the skim milk portion, the resultant milk is checked for its cholesterol content using the method mentioned in Example 1. The resultant milk is also checked for its milk fat and milk protein concentrations using a method known as the semi-microkjeldahl method. The results are listed in Table 6.

As Control 1, homogenized milk is processed under the same conditions as those described above without adding β-cyclodextrin thereto. As Control 2, unhomogenized milk is processed under the same conditions as those described above without adding β-cyclodextrin thereto. The milk of Control 1 and Control 2 is checked for its cholesterol content and for its milk fat and milk protein concentrations. The results are listed in Table 6.

TABLE 6

β-cyclodextrin Amount and Speed of Centrifugation		Milk Contents		Reduction Ratio (%)	
		Milk Fat (%)	Milk Protein (%)	Final Cholesterol Content (mg/100g)	Reduction Ratio (%)
0.5 (w/v)%	2,000 r.p.m.	3.45	3.18	0.82	93.5
	4,000 r.p.m.	3.60	3.60	0.6	95.2
	6,000 r.p.m.	3.50	3.50	0.16	98.7
1 (w/v)%	2,000 r.p.m.	3.30	3.18	0.15	98.8
	4,000 r.p.m.	3.45	3.15	0	100.0
	6,000 r.p.m.	3.60	3.60	0	100.0
Control 1	6,000 r.p.m.	3.50	3.37	12.6	0
Control 2	6,000 r.p.m.	3.70	3.25	12.6	0

As indicated in Table 6, the milk fat and milk protein concentrations of the resultant milk is not significantly different from those of Control 1 and Control 2. As a result, it is believed that the resultant milk is comparable to those of Control 1 and Control 2 in terms of taste and nutrient.

EXAMPLE 7

Unhomogenized milk is heated to 40°C. The milk is then pressurized in a homogenizer marketed by APV, Denmark, under model number 1-94.158 to the pressure condition listed in Table 7 (see below) and is sprayed to break the fat globules contained therein. The milk is then checked for its fat globule size by using an optical microscope sold by NIKON, Japan, under the trademark "LABPHOT-2". More particularly, a sample of the pre-treated milk is placed on the optical microscope, and the optical microscope magnification is adjusted to a desired setting. A fat globule image is then taken by using a "CCD" camera marketed by SONY, Japan, as model number XC-711. Next, the image taken by the camera is processed by an image analyzer sold by MEDIA-CYBER-NETICS as model number PLUS CAPTURE 3.0 (BETA) to filter out images of particles other than fat globules. Thereafter, the size of the fat globules in the processed image is calculated using the magnification setting of the optical microscope. The calculated size of the fat globules is listed in Table 7.

After the measurement of the fat globule size of the pre-treated milk, 1 (w/v)% of β-cyclodextrin in powder form is added to the pre-treated milk. Next, the milk-cyclodextrin mixture is mixed at 500 r.p.m. for 10 minutes at 0°C and then centrifuged at 4°C for 1 minute at 6,500 r.p.m. After discarding the cholesterol-cyclodextrin inclusion complexes and mixing the cream portion with the skim milk portion, the resultant milk is checked for its cholesterol content using the process described in "High Performance Liquid Chromatographic Analysis of Cholesterol in Milk", J. Dairy Science 66:2192-2194 (1983), which is incorporated herein by reference. The resultant milk is also checked for milk fat and milk protein

concentrations using the method mentioned in Example 6. The results are listed in Table 7.

As Control 1, unhomogenized milk, without performing the pre-treatment step of the present invention and without the application of β-cyclodextrin thereto, is processed under the same conditions as those mentioned above. As Control 2, unhomogenized milk, without performing the pre-treatment step of the present invention but with the application of β-cyclodextrin thereto, is processed under the same conditions as mentioned above. The results relating to Control 1 and Control 2 are listed in Table 7.

TABLE 7

Pressure (Kg/cm^2)	Mean Size of Fat Globules (μm)	Size of Fat Globules (μm)	Milk Fat (%)	Milk Protein (%)	Cholesterol Content (mg/100g)	Reduction Ratio (%)
Control 1	3.71	1-18	3.7	3.3	12.6	0
Control 2	3.71	1-18	3.8	3.5	8.56	32
25	2.43	1-15	3.8	3.4	5.92	53
50	2.08	1-10	3.7	3.4	3.40	73
75	1.68	1-7	3.8	3.5	1.15	91
100	0.99	0.5-3	3.8	3.4	0.2	98
200	0.76	0.1-2	3.8	3.5	0.1	99

As can be seen in Table 7, when a 100 to 200 kg/cm^2 pressure is used, substantially all of the fat globules of the starting milk are reduced to a size ranging between 0.1 and 3 μm and to a mean size ranging between 0.76 and 0.99 μm. It has been surprisingly and unexpectedly observed that when the fat globules have a size equal to these sizes, a reduction ratio of at least 98% can be obtained, while preserving taste and nutrition.

EXAMPLE 8

3 kg of raw milk is pressurized in the homogenizer mentioned in Example 7 to the pressure condition listed in Table 8 (see below) and is then sprayed to break fat globules. Next, 1 (w/v)% of β-cyclodextrin is added to the pre-treated milk and is then mixed for 10 minutes at 0°C and 500 r.p.m. After removing cholesterol-cyclodextrin inclusion complexes from the milk, the milk is separated into cream and skim milk by a cream separator sold by BOULOGNE, France, under the trademark "ELECTEAM", part number 92100. The cream is checked for its cholesterol content using the method described in Example 7 and is weighed using a balance. The results are listed in Table 8.

Control milk, without performing the pre-treatment step of the present invention and without the application of β-cyclodextrin thereto, is processed under the same conditions as those described above. The results relating to the control milk are listed in Table 8.

TABLE 8

Pressure (Kg/cm^2)	Cholesterol Content (mg/100g)	Reduction Ratio (%)	Amount of Cream (g/1000g)	Yield of Cream Production (%)
Control	12.6	0	147	100
100	0.2	98	87	59
200	0.1	99	103	70

As indicated in Table 8, while the yield of cream production according to the present invention is reduced by about 30 to 40% compared to the control milk, cream having an extremely low cholesterol content is obtained.

It will be understood that the embodiments described herein are merely exemplary and that a person skilled in the art may make many variations and modifications without departing from the spirit and scope of the invention. All such variations and modifications are intended to be included within the scope of the invention as defined in the appended claims.

Claims:

1. A process for removing cholesterol contained in an emulsion of animal origin having fat globules, characterized by the steps of reducing the size of substantially all of the fat globules to a predetermined size; contacting the emulsion with a predetermined amount of cyclodextrin such that the cyclodextrin forms insoluble inclusion complexes with the cholesterol; and separating substantially all of said complexes from the emulsion, wherein said predetermined size is within a range sufficient to cause the removal of substantially all of the cholesterol from the emulsion in a single performance of said contacting step and said separating step.

2. The process of Claim 1, characterized in that said predetermined size is not greater than about 3 μm.

3. The process of Claim 2, characterized in that said reducing step includes the step of pressurizing the emulsion in a homogenizer to a pressure not less than about 100 kg/cm^2.

4. The process of Claim 3, characterized in that said predetermined size ranges from about 3 μm to about 0.1 μm.

5. The process of Claim 4, characterized in that said pressure ranges from about 100 kg/cm^2 and to 200 kg/cm^2.

6. The process of Claim 5, characterized in that said predetermined size ranges from about 2 μm to about 0.1 μm.

7. The process of Claim 6, characterized in that said pressure is about 200 kg/cm^2.

8. The process of Claim 7, characterized in that said reducing step includes the step of heating the emulsion to about 40°C.

9. The process of Claim 8, characterized in that said separating step is performed using a centrifuge rotating at a speed ranging from about 2000 r.p.m. to about 6000 r.p.m.

10. The process of Claim 9, characterized in that said separating step is performed for a time period ranging from about 1 minute to about 5 minutes.

11. The process of Claim 10, characterized in that the emulsion has a temperature ranging between about 4°C and about 25°C during said separating step.

12. The process of Claim 11, characterized in that said contacting step includes the step of mixing the emulsion with the cyclodextrin at a temperature ranging between about 0°C and about 20°C.

13. The process of Claim 12, characterized in that said mixing step is performed for a time period ranging between about 5 minutes and about 30 minutes using a stirrer rotating at a speed ranging between about 50 r.p.m. and about 500 r.p.m.

14. The process of Claim 13, characterized in that said reducing step, said contacting step and said separating step are performed in a single, continuous operation.

15. The process of Claim 1, characterized in that the emulsion is milk.

16. The process of Claim 15, further characterized by the step of processing the milk after said separating step so as to produce a final product.

17. The process of Claim 16, characterized in that said processing step includes the step of separating the milk into cream and skim milk, thereby obtaining cream having a low cholesterol content.

18. A process for removing cholesterol contained in an emulsion of animal origin having fat globules, characterized by the steps of determining whether substantially all of the fat globules have a predetermined size; contacting the emulsion with a predetermined amount of cyclodextrin such that the cyclodextrin forms insoluble inclusion complexes with the cholesterol; and separating substantially all of the complexes from the emulsion, wherein said predetermined size is within a range sufficient to cause the removal of substantially all of the cholesterol from the emulsion in a single performance of said contacting step and said separating step.

19. The process of Claim 18, further characterized by the step of reducing the size of substantially all of the fat globules to said predetermined size.

20. The process of Claim 19, further characterized by the step of repeating said reducing step if it is determined that substantially all of the fat globules do not have said predetermined size.

21. The process of Claim 20, characterized in that said determining step includes the step of measuring the size of at least some of the fat globules of the emulsion.

22. The process of Claim 21, characterized in that said predetermined size is not greater than about 3 μm.

23. The process of Claim 22, characterized in that said reducing step includes the step of pressurizing the emulsion in a homogenizer to a pressure not less than about 100 kg/cm^2.

24. The process of Claim 18, further characterized by the step of reducing the size of substantially all of the fat globules to said predetermined size if it is

determined that substantially all of the fat globules do not have said predetermined size.

25. The process of Claim 18, characterized in that said range is sufficient to cause a reduction of at least about 98% in the cholesterol content of the emulsion in a single performance of said contacting step and said separating step.

26. A cholesterol removing process, characterized by the steps of selecting an emulsion of animal origin having fat globules, substantially all of which have a predetermined size; contacting the emulsion with a predetermined amount of cyclodextrin such that the cyclodextrin forms insoluble inclusion complexes with the cholesterol; and separating substantially all of said complexes from the emulsion, wherein said predetermined size is within a range sufficient to cause a reduction of at least about 98% of the cholesterol content of the emulsion in a single performance of said contacting step and said separating step.

27. The process of Claim 26, characterized in that said emulsion is milk having fat globules, substantially all of which have a size not greater than about 3 μm.

INTERNATIONAL SEARCH REPORT

Inter ›nal application No.
PCT/KR 98/00304

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: A 23 L 1/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: A 23 L 1/015; A 23 C 7/04,9/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91/16 824 A1 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 14 November 1991 (14.11.91), claims; pages 1-4.	1-8,12-27
X A	WO 91/11 114 A1 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 08 August 1991 (08.08.91), claims; pages 2,3.	1,18,26 2-17,19-25,27
X A	US 5 232 725 A (H.RODERBOURG et al.) 03 August 1993 (03.08.93), claims.	1,18,26 2-17,19-25,27

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna	Irmler
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal application No.
PCT/KR 98/00304

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